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Avian Synaptopodin 2 (Fesselin) Inhibits Actomyosin Dissociation by ATP and Alters the Structure of Smooth Muscle Myosin Filaments

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Fesselin or avian synaptopodin 2 stimulates actin polymerization in a Ca^{2+}-calmodulin dependent manner. Fesselin binding to F-actin inhibits myosin S1 binding and yet fesselin binds with moderate affinity to smooth muscle myosin. These properties suggest that fesselin could tether actin and myosin together in an inactive complex as caldesmon does. This possibility was tested by observing the effect of fesselin on the rate of dissociation of actin-myosin by ATP in a stopped-flow device. Dissociation was measured by light scattering (a measure of particle size) and by pyrene actin fluorescence (a specific measure of actin-myosin binding). Fesselin reduced the multi-exponential rates of change of light scattering and pyrene fluorescence in concentration dependent manners. Each light scattering trace had a rapid initial transition that was not present in pyrene fluorescence traces. That rapid light scattering change was likely due to dissociation of filamentous myosin. The reduction in rate of that rapid process could mean that fesselin alters the structure and dissociation kinetics of smooth myosin filaments. We examined changes in smooth muscle myosin with electron microscopy. ATP caused dissociation of myosin filaments in both the presence and absence of fesselin. In the absence of ATP, fesselin increased the size of myosin filaments. Furthermore, the filaments were arranged in parallel arrays. These results indicate that fesselin cross-links myosin to actin and also organizes myosin filaments in solution.

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Vimentin Filament Assembly is Altered by Substrate Elastic Modulus

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Background: The cytoskeletal protein vimentin is involved in the determination of cell mechanical properties, shape and motile behavior. We are investigating how vimentin participates in the response to external mechanical cues.
Methods: Normal and mouse embryo fibroblasts (mEF) harvested from the vimentin-knockout mouse are grown on fibronectin-coated polyacrylamide gels (Fn-PAA) of 0.5-40 kPa. Protein expression levels are assayed by Western blotting, and vimentin network distribution by immunofluorescence. Rates of vimentin subunit turnover are assayed by fluorescence recovery after photobleaching (FRAP) experiments; and atomic force microscopy (AFM) is used to measure cells' elastic and viscoelastic-plastic properties.
Results: Vimentin protein expression levels do not change in response to substrate stiffness. Whereas FRAP results in other cell types - which are less phenotypically responsive to substrate stiffness - show no difference in the rate of vimentin subunit turnover across various substrates, early results suggest that subunit turnover increases when fibroblasts are grown on physiological-range stiffnesses (~6 kPa). Also, many short vimentin filaments and squiggles (<15 μm in length) are evident that appear not to be connected to the extended vimentin network, and vimentin is more detergent-soluble under these conditions. The responses (e.g. shape, motility, etc.) of vimentin-null fibroblasts confirm vimentin's participation in these processes across the range of substrate stiffnesses. Finally, vimentin-null mEF are less stiff than mEF on ~6 kPa Fn-PAA, and initial experiments show that whether vimentin makes cells softer or stiffer depends upon the substrate elastic modulus.
Conclusion: Vimentin is expressed at consistent levels across conditions that cause changes in cell stiffness, shape and motility, yet the response of vimentin-knockout cells to the same conditions demonstrates vimentin's role in response to mechanical stimuli. We hypothesize that modulation of vimentin's assembly state underlies its contribution to cell mechanics.
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Actin Dynamics within Single Dendritic Spine Investigated by Two Photon Fluorescence Correlation Spectroscopy during Synaptic Plasticity

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Dendritic spines are the major site of excitatory synaptic input to pyramidal neurons of the hippocampus. The activity-dependent changes in the structure of spine structure have been clearly related to learning and memory processes. Actin is known to regulate cell shape and motility and in mature neurons is highly concentrated within dendritic spines (1). Indeed, the integrity as well as the changes in the spine structure have been shown to depend on the actin cytoskeleton dynamics. However, how actin filament dynamics are regulated during activity-dependent structural changes at synapses is up to now largely unexplored. Our study, by combining fluorescence microscopy (FM) with two-photon fluorescence correlation spectroscopy (2P-FCS)(2), allows us to simultaneously monitor the morphological changes and the dynamic behavior of actin filaments within single dendritic spine of neurons expressing actin-eGFP before and after chemical induction of synaptic plasticity (cLTP). Analyzing the autocorrelation curves from the 2P-FCS measurements and fitting them with two components diffusion model provide us quantitatively results, which show that: (1) actin dynamics within spines are significantly altered upon C-LTP induced morphological changes, (2) the highly dynamic actin filament exhibit a. heterogeneous structural composition, and (3) the regulations of actin filaments in single dendritic spine are precisely controlled individually instead of being a globally homogeneous function.
1. Cingolani, L.A. & Goda, Y. Actin in action: the interplay between the actin cytoskeleton and synaptic efficacy. Nat Rev Neurosci 9, 344-56 (2008).
2. Bacia, K., Kim, S.A. & Schwille, P. Fluorescence cross-correlation spectroscopy in living cells. Nat Methods 3, 83-9 (2006).

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Microfluidics with In-Situ Small-Angle X-Ray Scattering: A Tool to Investigate the Neurofilament Self-Assembly Mechanism

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The use of microfluidic chips with in-situ small-angle X-ray scattering (SAXS), offers new interesting possibilities for the study of biomaterials under flow. In first place, the manipulation of fluids allows for an experimental control (e.g. rate of mixing, shear rate, concentration gradients, confinement) that has been previously unavailable, opening the possibility for new experiments. In second place, sample consumption is reduced to the microliter scale, allowing experiments with expensive and rare materials. In third place, the constant flow of material prevents radiation damage (critical for X-ray synchrotron radiation).
In this work, we describe an approach to probe the mechanism of self-assembly of mature neurofilaments from its individual protein subunits at physiological ratio (NF-L:NF-M:NF-H of 7:3:2). This process is believed to consist of a series of distinct steps in vitro, involving the formation of several intermediate structures, but its full description has not been reported so far (Janmey PA. et al., 2003, Curr. Opin. Colloid Interface Sci. 8:40-47).
The protein solution under strong denaturing conditions (4 and 8 M urea, which prevents the assembly process) is mixed under flow with physiological buffer, leading to a drop in the urea concentration to levels where filament formation is favored. The geometry of the chip allows time-resolved tracking of the assembly process along the main microchannel. The emergence of new structural features with time, typical of larger aggregates, compared to the initial unimer solutions, is observed.
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Salt-Responsive Liquid Crystal Hydrogels: Neurofilament Network Structure and Mechanical Modulation

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Neurofilaments (NFs), the class of intermediate filaments found in neurons, are one of three structural protein groups that collectively form the cytoskeletal network. The active structure that NFs assume in the cell, and that permits